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	Examiner		
Interview Summary	MARK STAPLES	1637	A
participants (applicant, applicant's representative, F	PTO personnel):		
	(3)		
MARK STAPLES	(4)		
<u>SANDY LIVNAT</u>			
06/05/09 (ended).			
Type: a) Telephonic b) Video Conference of Personal (copy given to: 1) applications	ce ant 2)∐ applicant's repri	esentative]	
c) Personal (copy given to: 1) Li apprint			
or demonstration conducted: OILJ	5 20 A 1877 X 2 2 2 2 2		
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Claim(s) discussed: 1, 2, 17, and 24-28.	007)		
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Application No.

Applicant(s)

Staples, Mark

From: Sandy Livnet (slivnet@verizon net)

Sent: Thursday, June 04, 2009 8:00 AM

To: Staples, Mark

Subject: 10/522,405 Prior to your "allowance conf" with SPE

Mark:

I'm conferencing with clients right now and we'll have proposed amended claim 1 to you within an hour or so

Sandy

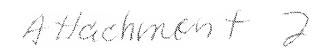
Sandy Livnat, Ph.D. Browdy and Neimark PLLC 624 Ninth Street, NW, Suite 300 Washington, DC 20001-5303

Tel: 202-628--5197 Fax: 202-737-3528

Email: slivnat@browdyneimark.com

(General email box: mail@browdyneimark.com)

CELL: 301-807-2803 Home: 301-588-0004



Staples, Mark

From:

Sandy Livnat (slivnat@verizon.net)

Sent:

Thursday, June 04, 2009 9:13 AM

To:

Staples, Mark

Cc:

Sandy (office); Livnat Home

Subject:

RE: 10/522,405 Prior to your "allowance conf" with SPE

Importance: High

Attachments: Proposed new amendments for Examiner 6-4-2009 pdf

Dear Examiner Staples:

Further to our discussion yesterday, I present to you proposed amendments to claim 1 (and parallel amendments to claim24), along with some minor changes in other dependent claims to maintain consistency. Please note the footnote on page 1 as regards the appearance markings of additions/deletions, informal use of bolding and highlighting and unofficial "claim identifers".

One of the main improvements is that we now refer to the "test sample" (preamble, etc.) vs. "control samples" introduced later in the claim.

As discussed, the claim now provides an "X axis" value for the determinations and ends with a clearer reference back to the preamble.

Other amendments re-order the way in which certain molecules or process steps are introduced in claim 1 that should make it simpler and clearer to follow.

We wish to re-emphasize that the novelty and non-obviousness of this invention lies in large part in the fact that NucSeql' and NucSeql' (and any additional standards that might be used, e.g., a NucSeqli', a NucSeqli', etc. – see various later claims) in the control sample are all localized on a single vector in which their ratio is known [See claim 1(2)(d)(i)]

The reason that relative CN is determined in claim 1 (rather than ending with the individual determinations in Claim 1(4) – as we touched upon yesterday - is that one distinguishing feature of this method is its improved accuracy over the prior art. Determining the ratio of NucSeql to NucSeql gets at that accuracy. Doing this extra step of division exploits the advantage of having the NucSeql' and NucSeql' on a single vector and in a known ratio to one another. Otherwise that would not have mattered. This provides the present invention with its improved accuracy as compared to using only the "concentrations" or "quantities" or "copy numbers" that are obtained in step (4).

Moreover, if it is known that the NucSeqII is always present in the starting material as, e.g., 2 copies per cell, then the absolute CN of NucSeqI can be calculated from the relative CN (see claim 2). Note that way in which the "absolute" and "relative" CN are now set out in the claims is a marked! improvement over the original claim set.

If this language (or something akin to it) is found acceptable, I suggest that it would be easier for you if we submitted a supplemental amendment rather than you doing all the work entailed in cranking out an Examiner's Amendment.

IF YOU RESPOND BY EMAIL, please use both my email addresses shown in the cc box.

Thank you.

Sandy Livnat

Sandy Livnat, Ph.D. Browdy and Neimark PLLC 624 Ninth Street, NW, Suite 300 Washington, DC 20001-5303

Tel: 202-628--6197 Fax: 202-393-1012

Email: slivnat@browdyneimark.com

(General email box: mail@browdyneimark.com)

Celt: 301-807-2803

(1)

PROPOSED NEW AMENDMENTS¹ (2009-June -04)

1. (proposed amended) A method of determining the relative copy number (CN) of a first nucleotide sequence I (NucSeqI) in a test sample using an amplification technique, said method comprising the steps of:

adding to the test sample that comprises NucSeqLand a chromosome-derived sectors
nucleofide sequence II (NucSeqII), the following ingredients:
mucleotides,
primers.
polymerase
a first probe specific directed to NucSeq1 and NucSeq1, comprising a first
fluorophore and a quencher, and/or and optionally, any additional reagents
required for amplification, wherein the sample comprises a chromosome derived
second-nucleatide sequence II (NucSeqil) and
a <u>second probe specific directed</u> to NucSeqII and NucSeqII ² comprising a second
floorophore and a quencher, wherein the first fluorophore and the second
fluorophore are different; and optionally
- any additional reagents required for amplification.

- carrying out the following amplification steps in one or more amplification cycles: (2)
 - (a.) amplifying NucSeqI in said test sample,
 - (b) amplifying NucScqII in said test sample.
 - in a control sample, to which said ingredients of (1) are added, amplifying at (0) multiple dilutions a third nucleotide sequence I' (NucSeqI'), corresponding to NucSeql and present in a control sample to which said first probe is also specific, at-multiple-dilutions in the presence of said first probe,

wherein the relationship of NocSeq! and NucSeq!' is defined as

³ Please note that additionaldeletions in the claims submitted on 5/26/09 have been incorporated (markings cleared). so that only proposed new amendments are shown as marked additions/deletions. Claim identifiers are "descriptive" and not listended to be "official" in this claim set. Bolding and highlighting is used "informally" to highlight certain additions that we discussed on 6/3/69

- (A) NucSeql hybridizes to the complement of NucSeql', and
- (B) NucSeql' hybridizes to the complement of NucSeql, both under stringent hybridization conditions, and, if NucSeql and NucSeql' differ in length, the shorter of the two is at most 30% shorter than the other; and
- (d) in a control sample, to which said ingredients of (1) are added, amplifying as multiple dilutions a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII and present in a control sample, at multiple dilutions to which said second probe is also specifie, in the presence of said second probe.

wherein the relationship of NucSeqII and NucSeqII' is defined as

- (A) NucSeqII hybridizes to the complement of NucSeqII', and
- (B) NucSeqII hybridizes to the complement of NucSeqII, both under stringent hybridization conditions, and, if NucSeqII and NucSeqII differ in length, the shorter of the two is, at most, 30% shorter than the other:

wherein

- (i) NucSeqI* and NucSeqII* are both localized on a single vector in which the ratio of NucSeqI* to NucSeqII* is known,
- (ii) standard curves SC₁ and SC₁₁ comprising at least two reference points are generated by amplification of NucSeqV and NucSeqIV. respectively, at multiple dilutions, and
- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;
- (3) determining the results of the amplifications of step (2) expressed as threshold cycle
 (Ct) as a function of quantity or concentration of the relevant amplified nucleotide sequence:
- (4) obtaining from the results in step (3) the following values:
 - "Conc-I_{SCI}" which is the concentration or quantity in the <u>test</u> sample of NucSoql determined from standard curve SC_I; and
 - (ii) "Cone-II_{SCII}" which is the concentration or quantity in the <u>test</u> sample of NucSeqII determined from standard curve SC_{II}, which standard curves express threshold cycle as a function of said concentration or quantity; and

(5) determining from the values obtained in step (4) the relative CN of NucSeql with respect to NucSeql by the formula:

thereby determining the relative CN of NucSeq1 in said test sample.

- 2. (proposed amended) A method for determining the absolute CN of a nucleotide sequence NucSeqI in a test sample, comprising:
 - (a) determining the relative CN using the method of claim 18, and
 - (b) multiplying the relative CN by the absolute CN of NocSeq! per cell.
- 3. (amended in last resp.) A method according to claim 1, wherein at least two different NucSeql' sequences, used for measuring a corresponding number of different NucSeql sequences, are localized on a single vector.
- 4. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqL and NucSeqL are the same.
- 5. (previously presented) A method according to claim 1 wherein the sequences of NucSeq11 and NucSeq11 are the same.
- 6. (amended in last Resp.) A method according to claim 2, wherein at least two different NucSeql' sequences, used for measuring a corresponding number of different NucSeql, are localized on a single vector.
- 7. (previously presented) A method according to claim 2 wherein the sequences of NucSeqL and the NucSeqL are the same.
- 8. (proviously presented) ———A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI are the same.
- 9. (previously presented) A method according to claim 6 wherein the sequences of NucSeq1 and the NucSeq1' are the same.
- 10. (praviously presented) ——A method according to claim 2 wherein the sequences of NucSeqΠ and the NucSeqΠ are the same.
- 11. (previously presented) ——A method according to claim 3 wherein the sequences of NucSeq11 and the NucSeq11 are the same.
- 12. (previously presunted) ——A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 13. (previously presented) ——A method according to claim 6 wherein the sequences of NucSeqU and the NucSeqU are the same.

- 14. (previously presented) A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 15. (previously presented) A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.
- 16. (previously presented) A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 17. (proposed amended) A method according to claim 1, wherein the test sample is derived from cells.
- 18. (previously presented) A method according to claim 17, wherein an absolute CN of NucSeq11 per cell is known.
- 19. (previously presented) A method according to claim 18, wherein at least two different NucSeql' sequences used for measuring a corresponding number of different NucSeql are localized on a single vector.
- 20. (previously presented) A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.
- 21. (previously presented) A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.
- 22. *(previously presented)* A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.
- 23. *(previously presented)* A method according to claim 20 wherein the sequences of NucSeq11 and the NucSeq11' are the same.
- 24. (proposed amended) A method of determining the relative CN of a first nucleotide sequence I (NucSeqI) in a <u>test</u> sample using an amplification technique, said method comprising the steps of:

(m)	adding to the test sample that comprises NucSeqL and a second nucleotide sequence I
	(NucSeqII), the following ingredients:
	nucleotides,
	primers,
	polymerase,
	a first probe specific directed to NucSeq1 and NucSeq1', comprising a fluorophore
	and a quencher, and optionally, any additional reagents required for amplification
	wherein the sample comprises a second maleoride sequence H (NucSeqH) and/or

a probe directed to NucSeqII and NucSeqII' comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different;

- any additional reagents required for amplification.

- (2) carrying out the following amplification steps in one or more amplification cycles:
 - (a) amplifying NucSeql in said test sample.
 - (b) amplifying NucScall in said test sample.
 - in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeql'), corresponding to NucSeql-and-present in a control-sample, at multiple dilutions to which said first probe is also specific, in the presence of said first probe, wherein the relationship of NucSeql and NucSeql' is defined as
 - (A) NucSeqL by bridizes to the complement of NucSeqU, and
 - (B) NucSeql' hybridizes to the complement of NucSeql, both under stringent hybridization conditions, and, if NucSeql and NucSeql' differ in length, the shorter of the two is, at most, 30% shorter than the other;
 - (d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions, a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII and present in a control sample, at multiple dilutions to which said second probe is also specific, in the presence of said second probe, wherein the relationship of NucSeqII and NucSeqII' is defined as
 - (A) NucSeqII hybridizes to the complement of NucSeqII', and
 - (B) NucSeqII hybridizes to the complement of NucSeqII, both under stringent hybridization conditions, and, if NucSeqII and NucSeqII differ in length, the shorter of the two is at most 30% shorter than the other;

wherein

- (i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of NucSeqI' to NucSeqII' is known,
- (ii) standard curves SC₁ and SC₀ comprising at least two reference points are generated by amplification of NucSeq1' and NucSeq1', respectively, at multiple dilutions.
- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and

- (3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of quantity or concentration of the relevant amplified nucleotide sequence;
- (4) obtaining from the results in step (3) the following values:
 - (i) "Cone-I_{SO}" which is the concentration or quantity in the sample of NucSeq! determined from standard curve SC_G and
 - "Cone-II_{SCII}" which is the concentration or quantity in the sample of NocSeqII determined from standard curve SC_{II}; and
- (5) determining from the values obtained in step (4) the relative CN of NucSeql with respect to NucSeql by the formula:

thereby determining the relative CN of NucSeq1 in said test sample.

- 25. (new: proposed amended) The method of claim 1 wherein the quantity in the <u>test</u> sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.
- 26. (new: proposed amended) The method of claim 1 wherein the concentration in the <u>test</u> sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.
- 27. (now) proposed amended) The method of claim 24, wherein the quantity in the <u>test</u> sample in step (4) is the number of copies of NucSeq1 or NucSeq1 obtained from the respective standard curves in which the quantity or relative dilution of NucSeq1' or NucSeq1', expressed as copy number, is plotted on the X-axis.
- 28. (now) (proposed amended)) The method of claim 24, wherein the concentration in the test sample in step (4) is the molar or weight concentration of NucSeq1 or NucSeq1) obtained from the respective standard curves in which the concentration or relative dilution of NucSeq1 or NucSeq1' is plotted on the X-axis.

ATTACHMENT 3

Staples, Mark

From:

Sandy Livnat (slivnat@verizon.net) Thursday, June 04, 2009 9:17 AM

Sent: To:

Staples, Mark

Co:

Sandy (office), Livnat Home

Subject:

RE: 10/522,495 Prior to your "allowance conf" with SPE

Importance: High

Oh yes., and if you need to reach me by phone today, please use my cell phone no. 301-807-2803 as I'm not sure where I will be when.

Thanks

Sandy

From: Sandy Livnat [mailto:slivnat@verizon.net]

Sent: Thursday, June 04, 2009 9:13 AM

To: 'mark.staples@uspto.gov' Cc: Sandy (office); Livnat Home

Subject: RE: 10/522,405 Prior to your "allowance conf" with SPE

Importance: High

Dear Examiner Staples:

Further to our discussion yesterday, I present to you proposed amendments to claim 1 (and parallel amendments to claim24), along with some minor changes in other dependent claims to maintain consistency. Please note the footnote on page 1 as regards the appearance markings of additions/deletions, informal use of bolding and highlighting and unofficial "claim identifers".

One of the main improvements is that we now refer to the "test sample" (preamble, etc.) vs. "control samples" introduced later in the claim.

As discussed, the claim now provides an "X axis" value for the determinations and ends with a clearer reference back to the preamble

Other amendments re-order the way in which certain molecules or process steps are introduced in claim 1 that should make it simpler and clearer to follow.

We wish to re-emphasize that the novelty and non-obviousness of this invention lies in large part in the fact that NucSeql' and NucSeqll' (and any additional standards that might be used, e.g., a NucSeqll', a NucSeqlV', etc. – see various later claims) in the control sample are all localized on a single vector in which their ratio is known [See: claim 1(2)(d)(i)]

The reason that relative CN is determined in claim 1 (rather than ending with the individual determinations in Claim 1(4) – as we touched upon yesterday – is that one distinguishing feature of this method is its improved accuracy over the prior art. Determining the ratio of NucSeql to NucSeql gets at that accuracy. Doing this extra step of division exploits the advantage of having the NucSeql and NucSeql! on a single vector and in a known ratio to one another. Otherwise that would not have

mattered. This provides the present invention with its improved accuracy as compared to using only the "concentrations" or "quantities" or "copy numbers" that are obtained in step (4).

Moreover, if it is known that the NucSeqII is always present in the starting material as, e.g., 2 copies per cell, then the absolute CN of NucSeqI can be calculated from the relative CN (see claim 2). Note that way in which the "absolute" and "relative" CN are now set out in the claims is a markedI improvement over the original claim set.

If this language (or something akin to it) is found acceptable, I suggest that it would be easier for you if we submitted a supplemental amendment rather than you doing all the work entailed in cranking out an Examiner's Amendment.

IF YOU RESPOND BY EMAIL, please use both my email addresses shown in the cc box.

Thank you.

Sandy Livnat

Sandy Livnat, Ph.D. Browdy and Neimark PLLC 624 Ninth Street, NW, Suite 300 Washington, DC 20001-5303 Tel: 202-628--5197

Fax: 202-393-1012

Email: slivnat@browdyneimark.com

(General email box: mail@browdyneimark.com)

Cell: 301-807-2803

ATTACH MENT Y

Staples, Mark

From: Sandy Livnat (silvnat@verizon.net)

Sent: Friday, June 05, 2009 8:29 AM

To: Staples, Mark

Cc: Sandy (office); Mary Anne Kornbau

Subject: RE: 10/522,405 Question

Mark:

I just finished conferring with clients. I need to talk to you about one issue in the about-to-be-amended claim language.

Since it doesn't appear to make sense to start "exchanging paper", it would best be handled in a brief phone call.

I can phone you now.... would this be an OK time? Or if you prefer to phone me, I'm currently at 301-588-0004

Thanks

Sandy

Sandy Livnat, Ph.D. Browdy and Neimark PLLC 624 Ninth Street, NW, Suite 300 Washington, DC 20001-5303

Tel: 202-628--5197 Fax: 202-393-1<mark>01</mark>2

Email: slivnat@browdyneimark.com

(General email box: mail@browdyneimark.com)

CELL: 301-807-2803 Home: 301-588-0004



Staples, Mark

From: Sandy Livnat [slivnat@verizon.net]

Sent: Friday, June 05, 2009 10:50 AM

To: Staples, Mark

Cc: Sandy (office); Mary Anne Kombau

Subject: 10/522,405 PROPOSED CLAIMS and Remarks re: Support (Our Ref. Cossarizza-1)

Importance: High

Attachments: Claims - Proposed Amds (and support) for entry by Examiner's Amd (2009-06-05).doc

Mark

Attached please find the proposed amendments we discussed and additional remarks regarding support for them.

As I understand it, this proposed claim set will be attached to an interview summary you write up, and will be "converted" into an examiner's amendment that enters the claims into the record prior to allowance.

Please contact me with any further questions or comments.

Thank you

Sandy

Sandy Livnat, Ph.D. Browdy and Nelmark PLLC 624 Ninth Street, NW, Suite 300 Washington, DC 20001-5303

Tel: 202-628--6197 Fax: 202-383-1012

Email: slivnat@browdyneimark.com

(General email box: mail@browdyneimark.com)

CELL: 301-807-2803 Home: 301-888-0004

PROPOSED NEW AMENDMENTS¹ (2009-June -05)

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nucleoti	ide sugui	nce I (NucSegI) in a <u>test</u> sample using an amplification technique, saul method		
compris	ing the s	repri of	,·····	
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(2)		cartying out the following amplification steps in one or more amplification cycles:		Deleted: and optionals any
	(a)	amplifying NucSeqLin said test sample.		additional magents required for amplification, wherein the sample
	(b)	amphfying NucSeqII in said test sample,		comprises a abromo como de rived second assilvation sequence (I
	(0)	in a control samule, to which said ingredients of (1) are added, amplifying at		(NucSeqti) and
		multiple dilutions a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI to which said first probe is also specific, in the presence of said first		Coleted *
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Marking of claim amendments and use of claim identifiers are relative to the amended claims submitted in the Response of \$7267,009, which, according to the Examiner, will be emered first. Therefore, all markings from those ewher filed claims have been removed - and those earlier claims and all consulered to be "previously presented".

(4)

second probe.

- (A) NucSeqLhybridizes to the complement of NucSeqL, and
- (B) NucSeql' hybridizes to the complement of NucSeql, both under stringent hybridization conditions, and, if NucSeql and NucSeql' differ in length, the shorter of the two is at most 30% shorter than the other and

in a control sample, to which said ingradients of (1) are added, amplifying at multiple delations a fourth nucleotide sequence If (NucSeqIP) corresponding to NucSeqII to which said accord probe is also specific. In the presence of and

wherein the relationship of NucScott and NucScutt' is defined as

- (A) NucSeq11 hybridizes to the complement of NucSeq11', and
- (B) NucSeqU hybridizes to the complement of NucSeqU, both under stringent hybridization conditions, and, if NucSeqU and NucSeqU differ in length, the shorter of the two is, at most, 30% shorter than the other;

wherein

- (i) NucSeqP and NucSeqIP are both localized on a single vector in which the "" ratio of NucSeqIP to NucSeqIP is known,
- (ii) standard curves SC, and SC_{ii} comprising at least two reference points are generated by amplification of NicSeq1' and NicSeq1', respectively, at multiple dilutions, wherein the starting quantity, commentation or dilution of NicSeq1' and NicSeq11' is known, and
- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;
- (3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of said marting quantity, concentration or dilution;
- (4) obtaining from the results in step (3) the following values:
 - (i) "Cone-Ise?" which is the concentration, [[or]] quantity or dilution in the jest sample of NucSeq) determined from standard curve SCz and

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- (ii) "Conc-H_{SCH}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeq1I determined from standard curve SC_{th} which standard curves express threshold cycle as a function of said <u>starting</u> concentration, [[or]] quantity or dilution; and
- determining from the values obtained in step (4) the relative CN of NucSeq1 with respect

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 to NucSeq1 by the formula:

Relative CN = Conc-I_{SCI}

Conc-I_{SCII}

thereby determining the relative CN of Not Segt in said test sample.

- 2. (currently amended) A method for determining the absolute CN of a nucleoside sequence NucSeq1 in a jest sample, comprising:
 - (a) determining the relative CN using the method of claim 18, and
 - (b) multiplying the relative CN by the absolute CN of blueSeqfI per cell.
- 3. **Iperconnly presented: A method according to claim 1, wherein at least two different NucSeql' sequences, used for measuring a corresponding number of different NucSeql sequences, are localized on a single vector.
- previously presented; A method according to claim 1 wherein the sequences of NucSeq1 and NucSeq1 are the same.
- 5. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII are the same.
- 6 apreviously presented)——A method according to claim 2, wherein at least two different. NucSeq! sequences, used for measuring a corresponding number of different NucSeq!, are localized on a single vector.
- 7. *(previously presented)* A method according to plain: 2 wherein the sequences of NucSeq1 and the NucSeq1 are the same.

- (previously prevented) ——A method according to claim 3 wherein the sequences of NucSeq1 and the NucSeq1 are the same.
- (previously presented) A method according to claim 6 wherein the sequences of NucSent and the NucSent' are the same.
- (previously presented) A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII are the same.
- (previously presented) A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII are the same.
- (previously presented) A method according to claim 4 wherein the sequences of NucSeq11 and the NucSeq11 are the same.
- 13. (previously presented) A method according to claim 6 wherein the sequences of NucSeqff and the NucSeqff are the same.
- 14. (previously presented) A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII are the same.
- (previously presented) A method according to claim 8 wherein the sequences of NucSeqli and the NucSeql' are the same.
- 16. (previously presented) A method according to claim 9 wherein the sequences of NucSeq11 and the NucSeq11' are the same.
- 17. (currently amended) A method according to claim 1, wherein the <u>test</u> sample is derived from cells
- (previously presented) A method according to claim 17, wherein an absolute CN of NecSeq11
 per cell is known.
- 19. *(previously presented)* A method according to claim 18, wherein at least two different NucSeql* sequences used for measuring a corresponding number of different NucSeql are localized on a single vector.

- 20. (previously presented) A method according to claim 18, wherein the sequences of NucSeq1 and the NucSeq1 are the same.
- Questionally presented: A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 22. (previously prevented) A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.
- (previously presented) A method according to claim 20 wherein the sequences of NucSeqH and the NucSeqH are the same.
- 24. (currently amounted) A method of determining the relative CN of a first nucleotide sequence 3 (NucSeqI) in a 3gg, sample using an amplification technique, said method comprising the steps of:
 - (1) adding to the fest sample that comprises NucSeqLand a second nucleonde sequence if (NucSeqI), the following ingredients.
 - nucleotides.
 -primers,
 - polymerase
 - a first probe specific to NucSeql, comprising a first fluorophore and a quencher, and/or a second probe specific to NucSeqll comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different; and optionally
 - : any additional reagents required for amplification,
 - (2) carrying out the following amplification steps in one or more amplification cycles:
 - (a) amplifying NucSeql in said (est sample,
 - (b) amplifying NucSeqII in said test sample.
 - (e) in a control sample, to which said ingredients of (1) are added, amplifying as multiple dilutions a third nucleotide sequence i' (NucSeql') corresponding to NucSeql to which said first probe is also specific, in the presence of said first probe.

wherein the relationship of NucSeq! and NucSeq!' is defined as

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- (A) NumSeql hybridizes to the complement of NucSeql', and
- (B) NucSeq!' hybridizes to the complement of NucSeq!, both under strongent hybridization conditions, and, if NucSeq! and NucSeq!' differ in length, the shorter of the two is at most 30% shorter than the other; and

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(d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dibutions a fourth uncleotide sequence B* (NucSeqB*) corresponding to NucSeqB to which said second probe is also specific, in the presence of said 120 pt. Desce After: 6 90. Car ensuing: 1.5 77mm

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wherein the relationship of NecSeqH and NucSeqH is defined as

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sample, at multiple dilutions.

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- (A) NucScaff hybridizes to the complement of NucScaff', and
- (B) NucSeqII' hybridizes to the complement of NucSeqII, both under stringent hybridization conditions, and, if NucSeqII and NucSeqII' differ in length, the shorter of the two is, at most, 30% shorter than the other:

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wherein.

second probe.

(i) NucSeqf' and NucSeqff' are both localized on a single vector in which the ratio of NucSeqff' to NucSeqff' is known. Sonmanted: Louent: Lain: 90 pt. line (pacing: 7:11:12:00

(ii) standard curves SC₁ and SC₁₁ comprising at least two reference points are generated by amplification of NucSeql' and NucSeql', respectively, at multiple dilutions, wherein the starting quantity, concentration or dilution of NucSeql' and NucSeql' is known, and

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- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;
- (3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of said startion quantity, concentration or dilution:
- (4) obtaining from the results in step (3) the following values:

(i) "Cone-l₁₀₀" which is the concentration, [[or]] quantity or <u>dilution</u> in the <u>test</u> sample of NucSeq! determined from standard curve SC₁; and Normathed: 14-vel 1, line vascing. 1.5 lines, Koop with such

- (ii) "Cone-Hscn" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqII determined from standard curve SCn, which standard curves express threshold cycle as a function of said starting concentration, [[or]] quantity or dilution; and
- (5) determining from the values obtained in step (4) the relative CN of NucSeq1 with respect to NucSeq1 by the formula:

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thereby determining the relative CN of NucSequin said test sample.

- 25. (currently amended) The method of claim 1 wherein the quantity in the <u>1831</u> sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI or NucSeqII, expressed as copy number, is plotted on the X-axis.
- 26. (currently amended) The method of claim 1 wherein the concentration in the <u>lest</u> sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.
- 27. (currently amended) The method of claim 24, wherein the quantity in the test sample in step (4) is the number of copies of NucSeq1 or NucSeq1 obtained from the respective standard curves in which the quantity or relative dilution of NucSeq1' or NucSeq1', expressed as copy number, is plotted on the X-axis.
- 28 (currently amended) The method of claim 24, wherein the concentration in the <u>test</u> sample in step (4) is the molar or weight concentration of NucSeql or NucSeql obtained from the respective standard curves in which the concentration or relative dilution of NucSeql' or NucSeql' is plotted on the X-axis.

REMARKS FOR PROPOSED NEW AMENDMENTS

As discussed with the Examiner by telephone interview on June 3, 4 and 5, 2009, Applicants have made the above amendments which, upon entry via Examiner's Amendment, are believed to place the case in condition for allowance. Several of the amendments are discussed in more detail below. Amendments to claim 1 have been introduced in parallel into claim 24 (with the one difference that in claim 1, NucSeqH is chromosome-derived). Many of the amendmenta involved rearrangement of subject matter present in the original (and earlier) claims and are thereby supported. Therefore, no new matter has been added by any of the presently proposed amendments.

Starting, Quantity Concentration or Dilution

At a number of places in claim 1. Applicants have indicated that the X axis of the standard curves is the "starting" quantity, concentration or dilution. The corresponding values for the unknowns are obtained by interpolation of these curves on the way to determining relative CN of NucSeql. Support in the specification for the amended language is as follows (emphasis added):

Page 1, lines 15-29

- a third nucleotide sequence l'... present in a control sample is simplified at various dilutions, and
- d) a fourth medicatide sequence W... present in a control sample is amplified at various dilutions.

where the ratio of the concentrations of nucleotide sequence Γ and Π' is known where the amplifications of ... nucleotide sequences Γ and Π' in various dilutions allows standard curves ... to be made, the concentrations of I and II are determined by using the respective standard curve SC_k and the relative concentrations allows the relative copy number CN of sequence I (versus nucleotide sequence II) to be determined ...

Page 4, lines 24-26

It is also very easy to determine the DNA concentration and benez the copy number of the markentide sequence per volume.

Page 9, lines 26-21;

The standard curves were made by introducing a **known number** of copies of vector per well.

Page 9, lines 2-3;

The absolute concentration of the controls was done using limiting dilution assays"

Figures 1-4 -- labeling of X-axis

For each figure, the X axis is labeled "Log Starting Quantity, copy number"

"Test" sample

Support for use of "test" sample in claim 1 and various dependent claims is present in multiple locations throughout the specification.

"Control sample"

Support for "control sample" or "control" in claim 1(2)(e) and 1(2)(d) is found at least in the cities from page 1 and 9 presented above.

Staples, Mark

ATTACHMENT

From:

Sandy Livnat (slivnat@verizon.net)

Sent

Friday, June 05, 2009 1:07 PM

To:

Staples, Mark

Co:

Mary Anne Kombau, Sandy (office)

Subject:

RE: 10/522,405 PROPOSED CLAIMS and Remarks re: Support (Our Ref. Cossarizza-1)

Attachments: Claims - Proposed Amds (and support) for entry by Examiner's Amd (2009-06-05).pdf

Mark:

Fret not! The sidebar annotations, colors, etc. can be "disappeared" by resetting the "review functions" of Track Changes in MS Word. This is how we do it for all amendments. [the only thing not affected are the double brackets which are OK appearance-wisel

- Open the Track Change Bar by right clicking in your tool bar/menu bar. area on top.
- Checkmark "Reviewing" in the box that opens this will open the Reviewing Tool Bar.
- Left click the drop-down arrow next to "Show" and select Options. which opens up a dialog box: make the following settings

"Underline color" --- "Automatic"

"Strikethrough color"---"Automatic"

"Formatting" --- "None"

"Changed lines" --- "None"

("Comments color" - irrelevant as they're not showing)

"Use Ralloons" -- NEVER

It should now look exactly like what you seek.

I've converted mine to pdf and attached it here too - should look exactly like the Word version on my screen (and your screen with the above settings). Or you can simply print out the pdf version for phsylical attachment to an Examiner's amendment (or for saving on your system).

We use this approach rather than "hard" underscoring" and "hard" strikethrough" because by "accepting all changes" you now have clean amended claims (other than manual cleanup of bracketed stuff) ready to work on for a subsequent amendment....

Let me know how these two options work. Otherwise, if you must have a Word version with "hard" underscoring and strikethroughs,, it will take a secretaray a few hours to "get to it" and to "do it".

Thanks Sandy

From: Staples, Mark [mailto:Mark.Staples@USPTO.GOV]

Sent: Friday, June 05, 2009 12:50 PM

To: Sandy Livnat

Subject: RE: 10/522,405 PROPOSED CLAIMS and Remarks re: Support (Our Ref:

Cossarizza-1)

Please send me a copy of the amended claims in a MS Word document all in black font and without any sidebar annotations. Please keep the underlining and strikethroughs as appropriate for claim amendments.

Thank you,

Mark Staples
Patent Examiner
United States Patent and Trademark Office
Art Unit 1637
Patent Hoteling Program
Mail Stop Remsen 2D18
(571) 272-9053

From: Sandy Livnat [mailto:slivnat@verizon.net]

Sent: Friday, June 05, 2009 10:50 AM

To: Staples, Mark

Cc: Sandy (office); Mary Anne Kornbau

Subject: 10/522,405 PROPOSED CLAIMS and Remarks re: Support (Our Ref:

Cossarizza-1)

Importance: High

Mark:

Attached please find the proposed amendments we discussed and additional remarks regarding support for them.

As I understand it, this proposed claim set will be attached to an interview summary you write up, and will be "converted" into an examiner's amendment that enters the claims into the record prior to allowance.

Please contact me with any further questions or comments.

Thank you

Sandy

Sandy Livnat, Ph.D. Browdy and Neimark PLLC 624 Ninth Street, NW, Suite 300 Washington, DC 20001-5303

Tel: 202-628--5197 Fax: 202-393-1012

Email: slivnat@browdyneimark.com

(General email box: mail@browdyneimark.com)

CELL: 301-807-2803 Home: 301-588-0004

PROPOSED NEW AMENDMENTS¹ (2009-June -05)

1. (currently amended) A method of determining the relative copy number (CN) of a first nucleotide sequence I (NucSeqI) in a <u>test</u> sample using an amplification technique, said method comprising the steps of:

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(1)	adding to the test sample that comprises NucSeqLand a chromosome-derived second
	nucleotide sequence II (NucSeqII), the following ingredients:
	nucleotides,
	primers,
	polymerase
	a <u>first probe specific directed-to NucSeqI and NucSeqI</u> , comprising a first
	fluorophore and a quencher, and/or and optionally, any additional reagonts required
	for amplification, wherein the sample comprises a chromosome-derived accord
	nacicotide sequence II (NacSeqII) and
	a second probe specific directed to NucScqII and NucScqIF-comprising a second
	fluorophore and a quencher, wherein the first fluorophore and the second fluorophore
	are different; and optionally
	- any additional reagents required for amplification.

- any manananaricasemo required na ampinicanali.
- (2) carrying out the following amplification steps in one or more amplification cycles:
 - (a) amplifying NucSeql in said test sample,
 - (b) amplifying NucSeqII in said test sample.
 - (c) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI and present in a control sampleto which said first probe is also specific, at multiple dilutions in the presence of said first probe.

wherein the relationship of NucSeqI and NucSeqI' is defined as

Marking of claim amendments and use of claim identifiers are relative to the amended claims admirted in the Response of 5/26/2009, which, according to the Examiner, will be entered first. Therefore, all markings from those earlier filed claims have been removed - and those earlier claims are all considered to be "previously presented".

- (A) NucSeql hybridizes to the complement of NucSeql*, and
- (B) NucSeql' hybridizes to the complement of NucSeql, both under stringent hybridization conditions, and, if NucSeql and NucSeql' differ in length, the shorter of the two is at most 30% shorter than the other, and
- (d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a fourth nucleotide sequence II* (NucSeqII*) corresponding to NucSeqII and present in a control sample, at multiple dilutions to which said second probe is also specific, in the presence of said second probe.

wherein the relationship of NucSeqII and NucSeqII' is defined as

- (A) NucSeqII hybridizes to the complement of NucSeqII*, and
- (B) NucSeqII hybridizes to the complement of NucSeqII, both under stringent hybridization conditions, and, if NucSeqII and NucSeqII differ in length, the shorter of the two is, at most, 30% shorter than the other:

wherein

- (i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of NucSeqI' to NucSeqII' is known.
- (ii) standard curves SC₁ and SC₂ comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions, wherein the starting quantity, concentration or dilution of NucSeqI' and NucSeqII' is known, and
- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single comainer and monitored by fluorescence during amplification;
- (3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of said starting quantity, concentration or dilution;
- (4) obtaining from the results in step (3) the following values:
 - (i) "Conc-I_{SCI}" which is the concentration, [[or]] quantity or dilution in the <u>test</u> sample of NucSeq! determined from standard curve SC₁; and

- (ii) "Conc-II_{SCR}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqII determined from standard curve SC_R, which standard curves express threshold cycle as a function of said starting concentration, [[or]] quantity or dilution; and
- (5) determining from the values obtained in step (4) the relative CN of NucSeql with respect to NucSeql by the formula;

thereby determining the relative CN of NucSoqI in said test sample.

- 2. (currently amended) A method for determining the absolute CN of a nucleotide sequence NucSeq1 in a test sample, comprising:
 - (a) determining the relative CN using the method of claim 18, and
 - (b) multiplying the relative CN by the absolute CN of NucSeqII per cell.
- 3. (previously presented) A method according to claim 1, wherein at least two different NucSeq1' sequences, used for measuring a corresponding number of different NucSeq1 sequences, are localized on a single vector.
- 4. (previously presented) A method according to claim 1 wherein the sequences of NucSeq1 and NucSeq1' are the same.
- 5. (previously presented) A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII* are the same.
- 6. (previously presented) A method according to claim 2, wherein at least two different NucSeql are localized on a single vector.
- 7. (previously presented) A method according to claim 2 wherein the sequences of NucSeq1 and the NucSeq1 are the same.

- 8. (previously presented) A method according to claim 3 wherein the sequences of NucSeqL and the NucSeqL are the same.
- (previously presented) A method according to claim 6 wherein the sequences of NucSeqLand
 the NucSeqLare the same.
- 10. (previously presented) A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII* are the same.
- (previously presented) A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 12. (previously presented) A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 13. (previously presented) A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 14. (previously presented) A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 15. (previously presented) A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 16. (previously presented) A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 17. (currently amended) A method according to claim 1, wherein the test sample is derived from cells.
- 18. (previously presented) A method according to claim 17, wherein an absolute CN of NucSeq11 per cell is known.
- 19. (previously presented) A method according to claim 18, wherein at least two different NucSeq1' sequences used for measuring a corresponding number of different NucSeq1 are localized on a single vector.

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- 20. (previously presented) A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.
- 21. (previously presented) A method according to claim 18 wherein the sequences of NocSeqf1 and the NucSeqf1 are the same.
- 22. (previously presented) A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 23. (previously presented) A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII are the same.
- 24. (currently amended) A method of determining the relative CN of a first nucleotide sequence I (NucSeqI) in a test sample using an amplification technique, said method comprising the steps of:
 - (1) adding to the <u>test sample that comprises NucSeqI and a second nucleotide sequence II</u>

 (NucSeqII), the following ingredients:
 - __nucleotides,
 - ___primers.
 - polymerase
 - a first probe specific directed to NucSeql and NucSeql', comprising a first fluorophore and a quencher, and/or and optionally, any additional reagents required for amplification, wherein the sample comprises a chromosome derived second nucleotide sequence II (NucSeqII) and
 - a <u>second probe specific</u> directed to NucSeqII and NueSeqII—comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different; and optionally
 - any additional reagents required for amplification.
 - (2) carrying out the following amplification steps in one or more amplification cycles:
 - (a) amplifying NucSeqLin said test sample.
 - (b) amplifying NucSeqII in said test sample,
 - (c) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeqI') corresponding to

NucSeql and present in a control sampleto which said first probe is also specific, at multiple dilutions in the presence of said first probe.

wherein the relationship of NucSeql and NucSeql' is defined as

- (A) NucSeql hybridizes to the complement of NucSeql*, and
- (B) NucSeql' hybridizes to the complement of NucSeql, both under stringent hybridization conditions, and, if NucSeql and NucSeql' differ in length, the shorter of the two is at most 30% shorter than the other; and
- (d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a fourth nucleotide sequence II' (NucSeqII') corresponding to NucSeqII and present in a control sample, at multiple dilutions to which said second probe is also specific, in the presence of said second probe.

wherein the relationship of NucSeqII and NucSeqII' is defined as

- (A) NucSeqII hybridizes to the complement of NucSeqII*, and
- (B) NucSeqII' hybridizes to the complement of NucSeqII, both under stringent hybridization conditions, and, if NucSeqII and NucSeqII' differ in length, the shorter of the two is, at most, 30% shorter than the other;

wherein

- (i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of NucSeqI' to NucSeqII' is known,
- (ii) standard curves SC₁ and SC₁₁ comprising at least two reference points are generated by amplification of NucSeql' and NucSeql', respectively, at multiple dilutions, wherein the starting quantity, concentration or dilution of NucSeql' and NucSeql' is known, and
- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;
- (3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of said starting quantity, concentration or dilution:

- (4) obtaining from the results in step (3) the following values:
 - (i) "Conc-I_{SCI}" which is the concentration, [[or]] quantity <u>or dilution</u> in the <u>test</u> sample of NucSeql determined from standard curve SC_I; and
 - (ii) "Cone-H_{SCII}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqII determined from standard curve SC_{II}, which standard curves express threshold cycle as a function of said <u>starting</u> concentration, [[or]] quantity or dilution; and
- (5) determining from the values obtained in step (4) the relative CN of NucSeq1 with respect to NucSeq1 by the formula:

thereby determining the relative CN of NucSeqI in said test sample.

- 25. (currently amended) The method of claim 1 wherein the quantity in the <u>test</u> sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.
- 26. (currently amended) The method of claim I wherein the concentration in the <u>test</u> sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.
- 27. (currently amended) The method of claim 24, wherein the quantity in the <u>test</u> sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.
- 28. (currently amended) The method of claim 24, wherein the concentration in the <u>test</u> sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqII or NucSeqII is plotted on the X-axis.

REMARKS FOR PROPOSED NEW AMENDMENTS

As discussed with the Examiner by telephone interview on June 3, 4 and 5, 2009, Applicants have made the above amendments which, upon entry via Examiner's Amendment, are believed to place the case in condition for allowance. Several of the amendments are discussed in more detail below. Amendments to claim 1 have been introduced in parallel into claim 24 (with the one difference that in claim 1, NucSeqII is chromosome-derived). Many of the amendments involved rearrangement of subject matter present in the original (and earlier) claims and are thereby supported. Therefore, no new matter has been added by any of the presently proposed amendments.

Starting, Quantity Concentration or Dilution

At a number of places in claim 1, Applicants have indicated that the X axis of the standard curves is the "starting" quantity, concentration or dilution. The corresponding values for the unknowns are obtained by interpolation of these curves on the way to determining relative CN of NucSeql. Support in the specification for the amended language is as follows (emphasis added):

Page 1, lines 15-29

- a third nucleotide sequence 1'...present in a control sample is amplified at various dilutions,
 and
- a fourth nucleotide sequence II'... present in a control sample is amplified at various dilutions,

where the ratio of the **concentrations** of nucleotide sequence Γ and Π' is known where the amplifications of ... nucleotide sequences Γ and Π' at various dilutions allows standard curves ... to be made, the **concentrations** of Γ and Π are determined by using the respective standard curve SC_0 , and the relative concentrations allows the relative copy number Γ of sequence Γ (versus nucleotide sequence Γ) to be determined ...

Page 4, lines 24-26

It is also very easy to determine the DNA concentration and hence the copy number of the nucleotide sequence per volume.

Page 9, lines 20-21:

The standard curves were made by introducing a known number of copies of vector per well.

Page 9, lines 2-3;

The absolute concentration of the controls was done using limiting dilution assays?

Figures 1-4 - labeling of X-axis

For each figure, the X axis is labeled "Log Starting Quantity, copy number"

"Test" sample

Support for use of "test" sample in claim 1 and various dependent claims is present in multiple locations throughout the specification.

"Control sample"

Support for "control sample" or "control" in claim 1(2)(c) and 1(2)(d) is found at least in the cites from page 1 and 9 presented above.